"Biological Activity of Silver Nanoparticles Synthesized by Endophytic fungi of *Maytenus emarginata* (Willd.) Ding Hou."

A

Synopsis

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By

Miss. Sayyad Shahim Fatima Karim
M.Sc., B.Ed., M.Phil.

Under the guidance of

Dr. B.D.Gachande
M.Sc., Ph.D., SET, B.Ed., D.M.L.T.
Associate Professor

Department of Botany

N.E.S. Science College, Nanded-431605

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"Biological Activity of Silver Nanoparticles Synthesized by Endophytic fungi of *Maytenus emarginata* (Willd.) Ding Hou."

1. A brief resume of proposed work /problem.

Fungal-mediated synthesis of silver nanoparticles (AgNPs) has recently emerged as a novel platform in the field of nanobiotechnology. As compared to bacteria, fungi are more advantageous because of fast growing rate, very easy to culture and easy to maintain in the laboratory. Nanotechnology provides a good platform to modify and develop nanostructures having promising applications in fields of medicine and agriculture. Silver nanoparticles are more effective because of their high surface area to volume ratio. So that, a large proportion of silver nanoparticles is in direct contact with the environment.

The use of endophytic fungi for production of nanoparticles remains untouched. So the present research focuses on studies on endophytic fungi of *Maytenus emarginata* (Willd.) Ding Hou, its molecular characterization and applications of biosynthesized silver nanoparticles for its biological activities such as antimicrobial, antioxidant, anti-diabetic, anticancer and plant growth promoters that will be helpful in medicine and agriculture.

2. Introduction to proposed research topic.

In recent years the development of resistant pathogenic strains and emergence of new disease causing agents has been increased, so that pathogenic resistance is one of the world's most pressing public healthcare problems leads to increasing the demand for new bioactive compounds in medicine, agriculture and industry. Therefore, it is necessary to design and develop a new strategy to overcome such type of new limitations raised in medical and agricultural sciences.

Since plants have been a major source of bioactive compounds for drug discovery. Recently the attentions of many researchers increased towards nanoparticles due to their wide applications in industries, agriculture and medicine.

Nanotechnology is an important emerging field of modern research helps in designing, synthesizing, and manipulation of particles structure ranging from 1-100 nm.

Nanotechnology has now started new applications to change our lives. Now a day's growing field of nanotechnology has wide applications in various sectors of environment, energy, healthcare, agriculture, and consumer goods. Therefore, it has developed new idea in the academic community, investors, governments and industries.

The applications of nanomaterials and nanoparticles are rapidly increasing in various fields. Metal nanoparticles have a high fraction of surface atoms and a high specific surface area. The unique physicochemical properties of nanoparticles, includes catalytic activity, electronic properties, optical properties, magnetic properties, antibacterial, anticancer, antioxidant and antidiabetic properties. They are gaining the interest of scientists for their novel method of synthesis.

Endophytes are found in a wide variety of plant tissue types such as roots, stems, leaves, tubers, buds, ovules, seeds, fruits, xylem and bark (Tan and Zou, 2001).

The study revealed that approximately, there are near to 3,00,000 plant species on earth having host to one or more endophytes, and many of them may colonize different hosts. Plant endophytic fungi are novel and important for production of natural bioactive compounds with their potential use in agriculture, medicine and industry.

Amongst fungi, not much work has been done on mycosynthesis of silver nanoparticles from endophytic fungi of different plants. A very few report such as *Penicillium* spp isolated from the medicinal plant *Centella asiatica* and *Curcuma longa* shows the antibacterial and antifungal activity (Devi, 2012, Dattu *et al.* 2014). Mycosynthesis of silver nanoparticles from endophytic fungi, *Penicillium* species of *Glycosmis mauritiana*, and its antioxidant, antimicrobial, anti-inflammatory and tyrokinase inhibitory activity (Govindappa *et al.* 2016). Endophytic fungi namely, *Pencillium* sp., *Alternaria* sp., *Aspergillus* sp and *Cladosporium* sp from different parts of *Calotropis procera* for antimicrobial and antioxidant activities mediated by extracellular Synthesized Silver nanoparticles (Debjani *et al.* 2016). Silver nanoparticles from endophytic fungi of Leaf samples of *Garcinia xanthochyumus* and *Aravae lanata* as a effective antibacterial agents (Sunkar and Nachiyar, 2013).

The present investigation focuses on biological activity of silver nanoparticles from endophytic fungi of medicinal plant *Maytenus emarginata* (Willd.) Ding Hou.

Maytenus emarginata (Willd.) Ding Hou is an evergreen wild shrub that tolerates various types of stresses of the desert and belongs to family Celastraceae. It is locally called as Hekalni / Hekal (Naik, 1998). It is used in traditional medicine as stimulant, male contraceptive, restorative, anti-tumor, anti-bacterial, anti-leukemic, insecticidal and insect repellent activities (Dubravkova *et al.* 1998). The root of *Maytenus emarginata* exhibit highest radical scavenging effect with IC50 = 49.32 µg/ml (Savita *et al.* 2011). The extract of the leaves of *Maytenus emarginata* has antidiabetic activity (Bishnoi *et al.* 2016).

There is very few report on endophytic fungi from *Maytenus emarginata* (Willd.) Ding Hou includes *Phyllosticta capitalensis* isolated by (Naveen *et al.* 2014), *Xylaria* (Meenavalli *et al.* 2013), *Pestalotiopsis* sp. from *Maytenus ilicifolia* a medicinal plant from Brazil (Deeksha *et al.* 2016).

There is no report on synthesis of endophytic fungal silver nanoparticles from *Maytenus emarginata* (Willd.) Ding Hou and its antimicrobial, antioxidant, antidiabetic anticancer and plant growth promoting activity.

A wide variety of physical and chemical methods to synthesize nanoparticles are in practice but their inherent flaws that include contamination from precursor chemicals, use of toxic solvents and generation of hazardous by-products (Sunkar and Nachiyar, 2012). The present study focuses on the biosynthesis of silver nanoparticles using on faction of microbes, the endophytic fungi as a green alternative to the chemical method.

Therefore, present investigation was aimed to isolate fungal endophytes from *Maytenus emarginata* (Willd.) Ding Hou and to synthesize the silver nanoparticles to evaluate for biological (antimicrobial, antioxidant, antidiabetic, anticancer and plant growth promotion) activity.

3. Survey or review of literature and development in the relevant subject.

Khabat *et al.* (2011) evaluated extracellular biosynthesis of AgNPs with the diameters in the range of 5-50 by *Trichoderma reesei.*

Bharathidasan and Panneerselvam (2012) Isolated endophytic fungi such as *Penicillium janthinellum, Aspergillus concius* and *Phomosis* sp. from *Suaeda monica, Avicennia marina* and *Rhizophora mucronata* leaf.

Devi *et al.* (2012) studied biosynthesis of silver nanoparticles from three endophytic fungi *Aspergillus tamari*, *Aspergillus niger* and *Penicillium ochrochloron* isolated from ethno-medicinal plant *Potentilla fulgens* L.

Meenavalli *et al.* (2013) isolated *Xylaria* endophytic fungus from the leaves of *Maytenus emarginata* collected from evergreen forest of the Western Ghats in Southern India and was screened for the production of some bioactive metabolites and extracellular enzymes.

Dattu *et al.* (2014) studied extracellular biosynthesis of silver nanoparticles (AgNps) by an endophytic fungus *Penicillium* sp. isolated from healthy leaves of *Curcuma longa*.

Shelar and Chavan (2015) observed synthesis of silver nanoparticles by *Trichoderma harzianum.*

Govindappa *et al.* (2016) observed silver nanoparticles synthesized by *Penicillium* species, an endophytic fungus of *Glycosis mauritiana*.

Netala *et al.* (2016) studied synthesis of silver nanoparticles (AgNPs) from endophytic fungal strain *Pestalotiopsis microspora* isolated from the leaves of *Gymnema sylvestre* and *Aspergillus versicolor* from the leaves of *Centella asiatica*.

Harshita and Sardul (2017) reported synthesis of silver nanoparticles by *Alternaria tenuissima* isolated from *Punica granatum*.

4. Objectives of the research and proposed hypothesis.

- Isolation of endophytic fungi.
- Molecular characterization of endophytic fungi.
- Biomass production and biosynthesis of silver nanoparticles.
- Characterization of silver nanoparticles.
- Evaluation of antibacterial activity of silver nanoparticles.
- Evaluation of antifungal activity of silver nanoparticles.
- Evaluation of antioxidant activity of silver nanoparticles.
- Evaluation of anti-diabetic activity of silver nanoparticles.
- Evaluation of anticancer activity of silver nanoparticles.
- Evaluation of plant growth promotion of silver nanoparticles.

5. Methodology to be used and adopted.

The molecular characterization of endophytic fungi and the characterization of silver nanoparticles will be done with the help of UV-Visible Spectroscopy, TEM, FTIR & XRD. Standard methods and procedures/techniques will be used for isolation and evaluation of antibacterial, antifungal, antioxidant, anti-diabetic, anticancer and Plant growth promotion of silver nanoparticles from endophytic fungi of *Maytenus emarginata* (Willd.) Ding Hou.

6. Importance of the study-

International status

Resistance in pathogens is a challenge in fields of pharmaceutical, agriculture and biomedicine. Pathogenic resistance profiles in fungal and bacterial pathogens lead to develop resistance and require multiple treatments of broad-spectrum antibiotics and fungicides, which are more effective and less toxic to human and plants. Therefore, development of antimicrobial compounds using silver nanoparticles from endophytic fungi of *Maytenus emarginata* is a priority area of research in the modern era that overcomes such type of new limitations raised in medical and agricultural sciences.

There are scanty reports on fungal endophytes of *Maytenus emarginata*. But there is no any report with special reference to silver nanoparticles and their effect on antibacterial, antifungal, antioxidant, anti-diabetic, anticancer and plant growth promoter.

Therefore, keeping this background information, there is a need to explore more endophytic fungi from all tissues of host plant *Maytenus emarginata* and to screen out their silver nanoparticles biosynthesizing potential to analyze their antibacterial, antifungal, antioxidant, antidiabetic, anticancer and plant growth promoter activities.

***National status**

At national level there is no report on endophytic fungi from *Maytenus emarginata* for biosynthesis of silver nanoparticles as an antifungal, antioxidant, antidiabetic, plant growth promotion and antibacterial property in medicine and agriculture against human and plant pathogen for controlling different diseases. The cost of such medicine will be low. It will be best alternative for chemotherapeutics.

***Regional status**

Present research work will be novel for this Maharashtra region that can be helpful to provide endophytic fungal diversity from *Maytenus emarginata*. The biosynthesis of silver nanoparticles will be helpful to control human pathogenic bacteria such as *Mycobacterium tuberculosis*, *Salmonella typhi*, *Vibrio cholarae*, *Staphylococcus aureus* and *E. coli in vitro*. It may be helpful to control growth of fungal phytopathogen such as *Fusarium oxysporum*, *Alternaria alternata*, *Colletotrichum capsci* and *Helminthosporium turcicum*.

***Scope of Study**

The research work first time provides endophytic fungi from *Maytenus emarginata* from Maharashtra region and may be help in new fungal taxa record from area. Green synthesized nanoparticles from endophytic fungi are cheap, clean, nontoxic and eco-friendly and completely safe, so it can be used extensively. The multi-resistant pathogens due to antigenic drifts or shifts are ineffectively managed with current medications. This resistance to medication by pathogens has become a serious problem in public health and therefore it is mandatory to develop new alternatives that will be comes in the form of silver nanoparticles from endophytic fungi will be help in medicine and agriculture.

7. Proposed work plan- with a tentative chapter scheme

The entire work will be divided into two parts:-

The first part of work would be fully devoted for isolation, molecular characterization, identification, biomass production and biosynthesis & characterization of extracellular silver nanoparticles from endophytic fungi.

The second part of work would be mainly on evaluation of extracellular silver nanoparticles as antibacterial, antifungal, antioxidant, anti-diabetic, anticancer and plant growth promoters.

8. Year wise plan of work and targets to be achieved

The research work will be carried out as per the given work plan.

Year 2017-2018:

- Collection of literature and reference work.
- Standardization of methods and procedures.

- Collection of plant material (*Maytenus emarginata*) from different localities.
- Isolation of endophytic fungi from different parts of of *Maytenus emarginata*.
- Molecular characterization of endophytic fungi.
- Biosynthesis of silver nanoparticles and their characterization.

Year 2018-2019:

- Evaluation of silver nanoparticles for their bactericidal activity against human pathogens.
- Evaluation of silver nanoparticles for their antifungal activity.
- Evaluation of silver nanoparticles for their antioxidant activity.

Year 2019-2020:

- Evaluation of silver nanoparticles for their anti-diabetic activity.
- Evaluation of silver nanoparticles for their anticancer activity.
- Evaluation of silver nanoparticles for their plant growth promoting activity.
- Compilation of experimental data, submission of final synopsis and thesis.

9. Expected outcome

The interference of endophytes with nanoparticles is relatively new and is expected to have significant impact. Fungal endophytes are reported to secrete diverse group of biomolecules extracellularly by using cell free filtrate.

The present research work is novel and may provide information regarding some new endophytic fungal isolates from *Maytenus emarginata*. These fungal isolates may produce extracellular silver nanoparticles, some of which may show its antibacterial, antifungal, antioxidant, anti-diabetic, anticancer and plant growth promotion activity in medicine and agriculture. This work may pave a way to develop therapeutic strategy from natural resources.

Nanoparticles may provide a good platform to modify and develop nanostructures having promising applications in various fields therefore endophytic fungi isolated from *Maytenus emarginata* may found to be good producers of AgNPs which may remain untouched for nanoparticles production apart from being rich sources of secondary metabolites and may improve the crop health.

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Signature

Dr. B.D.Gachande
Research Guide

Signature

Miss. Sayyad Shahim Fatima
Research Scholar